Inactivation of *Listeria innocua* on Frankfurters by Ultraviolet Light and Flash Pasteurization

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ABSTRACT: ***Listeria monocytogenes*, a psychrotrophic foodborne pathogen, is a recurring postprocess contaminant on ready-to-eat meat (RTE) products including frankfurters. Flash (Steam) Pasteurization (FP) and ultraviolet light (254 nm-UVC) has been shown to reduce levels of *L. monocytogenes* and *L. innocua* on frankfurters. In this study, the use of UVC light followed by FP to inactivate *L. innocua*, a nonpathogenic surrogate for *L.monocytogenes*, on frankfurters that contained sodium diacetate and potassium lactate (SDA/PL) in a pilot-plant setting was investigated. Application of UVC (1.0 J/cm^2), followed by FP (0.75 s steam/121 °C) resulted in inactivation of 3.19 log *L. innocua*, while application of UVC (4.0 J/cm^2), followed by FP (3 s steam/121 °C) resulted in inactivation of 3.89 log of *L. innocua*. A refrigerated storage study (8 °C) of frankfurters that contained SDA/PL that were treated with UVC followed by FP revealed the growth of *L. innocua* was inhibited for approximately 8 wk following application of the interventions. The use of UVC in combination with FP had little effect on frankfurter color and texture. The combination of UVC, FP, and SDA/PL was found to be an effective hurdle process for decontamination of frankfurter surfaces.**

Keywords: frankfurters, flash pasteurization, *Listeria innocua*, ultraviolet light

Introduction

Listeria monocytogenes is an occasional postprocess contaminant on ready-to-eat (RTE) meat products, including frankfurters, and a number of foodborne illness outbreaks have been attributed to *L. monocytogenes* (Mead and others 1999; U.S. FDA 2003; Anonymous 2006, 2008). In the United States, the incidence of listeriosis has stabilized since its low point in 2002 (Anonymous 2006). In Europe, the incidence of listeriosis has increased in many nations as reported in a recent Eurosurveillance report (Denney and McLauchlin 2008). *L. monocytogenes* is capable of growth at refrigerated temperatures and in high-salt environments, which allows it to proliferate during long-term cold storage (Smith 1996). Because of the high mortality rate (20% to 30%) associated with infection, *L. monocytogenes* is given zero tolerance in RTE meat products in the United States (USDA FSIS 1989; Gerba and others 1996). In the last 10 y (1999 to 2008), there have been numerous Class I recalls of frankfurters and sausages, not including other RTE meats, due to contamination with *L. monocytogenes* (USDA FSIS 2008).

Frankfurters surfaces are typically contaminated after the cooking process and prior to packaging. Postprocess interventions, either alone or in combination with antimicrobial compounds may be used to inactivate and prevent the proliferation *L. monocytogenes* on RTE meat products. UVC (254 nm) irradiation, which exerts its bactericidal effect by production of cyclobutane pyrimidine dimers and 6 to 4 photoadducts in the bacterial chromosome, is an FDA-approved intervention that can be used for the surface decontamination of foods (U.S. FDA 2001; Reardon and Sancar 2005). Flash Pasteurization (FP), a process that has recently been commercialized, uses short pulses of steam to decontaminate the surfaces of fine emulsion sausages such as frankfurters or bratwurst immediately before packaging (Murphy and others 2005a, 2005b, 2006; Sommers and others 2008a). Both potassium lactate (PL) and sodium diacetate (SDA) have been approved by the U.S. Food and Drug Administration (FDA) for use in RTE meat products (U.S. FDA 2000) and combinations of the two can inhibit the growth of *L. monocytogenes* on RTE meats during long-term refrigerated storage (Mbandi and Shelef 2001; Seman and others 2002; Sommers and others 2003a). In a recent study, Sommers and others (2008) found that FP (1.5 s steam, 121 °C) inactivated 2 log of *L. innocua* on the surfaces of frankfurter surfaces that contained SDA and PL, and that growth of surviving *L. innocua* was inhibited during a 2-mo refrigerated storage period at 4 °C. Similar results were obtained using *L. monocytogenes* when frankfurters containing SDA and PL were exposed to UVC Light, with a 3-log inactivation of *L. monocytogenes* being obtained following a 2-mo refrigerated storage period (Sommers and others 2009).

L. innocua has similar inactivation and growth profiles to *L. monocytogenes* in response to UVC light, FP, ionizing radiation, and SDA/PL when inoculated onto RTE meat surfaces (Sommers and others 2002, 2008; Sommers and Geveke 2006). Therefore, studies conducted in a food pilot plant setting using FP have used *L. innocua* cocktails as a nonpathogenic surrogate in place of *L. monocytogenes* (Kozempel and others 2000; Sommers and others 2002, 2008; Murphy and others 2005a, 2006). The purposes of this study were to: (1) determine the effect of the combinatorial use of UVC followed by FP to inactivate the *L. monocytogenes* surrogate *L. innocua* on the surface of frankfurters that contain SDA/PL; (2) to determine the growth potential of *L. innocua* on UVC-FP treated frankfurters that contained SDA/PL; and (3) to determine the effect of UVC-FP on frankfurter color and texture.

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Materials and Methods

Frankfurters

Freshly manufactured frankfurters were purchased from a local manufacturer. The frankfurters consisted of beef, pork, water, salt, flavoring, paprika, sodium phosphate, SDA (0.07%), PL (1.13%), sodium erythorbate, sodium nitrate, and were 25% fat. Frankfurters were stored at −20 °C, and thawed overnight in a refrigerator for experimentation the following day.

L. innocua

Three *L. innocua* strains (51742, 33090, 33091) were obtained from the American Type Culture Collection (Manassas, Va., U.S.A.). The strains were propagated on Tryptic Soy Agar (BD−Difco Laboratories, Sparks, Md., U.S.A.) at 37 °C and maintained at 0 to 2 °C until ready for use. Identity of *Listeria* was confirmed by gram stain followed by analysis with gram-positive identification (GPI) cards using the Vitek Automicrobic System (bioMerieux Vitek Inc., Hazelwood, Mo., U.S.A.). The *L. innocua* isolates were screened on sheep's blood agar plates and *Listeria* Chromogenic Agar for hemolysin and listeriolysin O activity and tested negative.

L. innocua propagation and inoculation

Each *L. innocua* strain was cultured independently in 30-mL Tryptic Soy Broth (Difco) in 50-mL sterile tubes at 37 °C (150 rpm) for 18 h. The cultures were then diluted in Butterfield's phosphate buffer (BPB; Applied Research Inst., Newtown, Conn., U.S.A.) as a mixture. Refrigerated frankfurters were then placed on a sterile surface, rolled in 1 mL of diluted inoculum, including the ends, to a final concentration of 10^5 CFU/g, and placed in a refrigerator for approximately 30 min prior to UV and FP treatments. The inoculation, 10^5 CFU/g, is relatively high in comparison to the relatively low numbers associated with naturally occurring contaminations of frankfurters (Gombas and others 2003). However, preliminary experiments revealed that we were unable to recover viable *L. innocua* at lower inoculations (10^2 to 10^3 CFU/g) that would typically represent the upper limit of naturally occurring contaminations.

UVC irradiation

A custom made UVC irradiator containing four 24-inch UVC emitting bulbs (Atlantic Ultraviolet, White Plains, N.Y., U.S.A.), in a planar array, constructed of stainless steel was used. The apparatus delivered a UVC dose of 10 mW/s/cm^2 as determined by a calibrated UVX Radiometer (UVP Inc., Upland, Calif., U.S.A.) at a distance of 20 cm from the bulbs. Frankfurters were placed on a platform that was 20 cm below the bulbs and exposed to UVC for various times. Four frankfurters were treated simultaneously. The frankfurters were irradiated by rotating them 90° × 4 times during the exposure to UVC. Therefore a frankfurter exposed to 4 J/cm^2 UVC received 4 × 1 J/cm^2 (100 second) exposures. UVC treatment did not increase the surface temperature of the frankfurters to greater than 20 °C as determined using a hand held infrared thermometer (Model 25625-40, Oaklon Inc., Vernon Hills, Ill., U.S.A.).

Flash pasteurization

To assess the effect of the FP inactivation process, the surface-inoculated frankfurters were loaded into open preformed trays at the inlet of the flash pasteurization prototype unit (Alkar-RapidPak, Lodi, Wis., U.S.A.) as a single layer of 4 frankfurters. The frankfurters were then exposed to steam treatments (121 °C) for 0.5, 1.5, or 3 s. The frankfurters were then placed in sterile polynylon bags (Uline Inc., Philadelphia, Pa., U.S.A.), which were stored in an ice–water bath for approximately 30 min prior to enumeration of *L. innocua*.

UVC followed by flash pasteurization

Frankfurters were transferred from the UV apparatus and subjected to flash pasteurization as described above within 2 min.

Recovery and plating of *L. innocua*

Following FP the samples were assayed for colony forming units (CFU's) by standard pour plate procedures. Fifty milliliters of sterile BPB were added to a polynylon bag (Uline Inc.) bag that contained 4 frankfurters and shaken manually for 1 min (Sommers and Thayer 2000). The samples were then serially diluted in BPB, using 10-fold dilutions, and 1-mL of diluted sample was pour-plated using Palcam Medium (BD-Difco Inc., Sparks, Md., U.S.A.). Two 1-mL aliquots were plated per dilution. The Palcam plates were then incubated for approximately 48 h at 37 °C prior to enumeration for (CFU). Each experiment was conducted independently 3 times ($n = 3$).

Storage study

The same inoculation and recovery procedures were used and described in the previous sections with the exception that frankfurter packages were vacuum-sealed (30 mB) using a Multivac A-300 Vacuum Packager (Kansas City, Mo., U.S.A.). Single layer packages of frankfurters were held at 7 to 8 °C for 8 wk. Frankfurter packs were sampled every 2 wk. Each experiment was conducted independently 3 times ($n = 3$).

Color analysis

Color analysis was performed using a Hunter Lab Miniscan XE Meter (Hunter Lab. Inc., Reston, Va., U.S.A.; Sommers and others 2003a). The meter was calibrated using white and black standard tiles. Illuminate D65, 10° standard observer, and a 2.5-cm port/viewing area were used. Results are from 3 independent experiments, with 3 readings taken per experiment.

Shear force

Cutting force of the frankfurters was measured using a Texture Technologies Corp. (Scarsdale, N.Y., U.S.A.) TA-XT2 texture analyzer. A TA-7 Warner–Bratzler blade was used with a test speed of 2 mm/s, 55 mm distance, and a 20 g auto-trigger (Sommers and others 2003a). Maximum shear force (g) results are from 3 independent experiments, with 3 readings taken per experiment.

Statistical analysis

Descriptive statistics and analysis of variance (ANOVA) were performed using the descriptive statistics package of MS Excel (Microsoft Corp., Redmond, Wash., U.S.A.).

Results and Discussion

The increased sensitivity of microorganisms to heat following exposure to UVC or ionizing radiation is not new. Sommers and Geveke (2006) demonstrated the increased sensitivity of *L. innocua* to a process closely related to FP, the vacuum–steam–vacuum process, following UVC. Sommers and others (2003b) demonstrated the increased thermal sensitivity of *L. monocytogenes* inoculated onto fine emulsion turkey sausage following exposure to gamma radiation. Kim and Thayer (1996) demonstrated the increased thermal sensitivity of *Salmonella* following exposure to ionizing radiation, caused by the increased thermolability of DNA following the induction of DNA strand breaks. Increased thermal sensitivity of

bacteria following exposure to UVC irradiation has also been reported (Tyrrell 1979). Like gamma radiation, UVC indirectly causes DNA strand breakage in bacterial, yeast, and human cells through active DNA repair processes (for example, incision of the DNA 5′ and 3′ of the DNA adduct by nucleotide excision repair enzymes) and inhibition of DNA replication (Bonura and Smith 1975; Tang and Ross 1985; Zuk and Zabarowska 1987; Noz and others 1996) and UVC radiation causes a reduction in the molecular weight of human, bacterial, and fungal chromosomes as a result of single- and double-stranded DNA breaks. Like ionizing radiation, the introduction of DNA strand breaks is the likely mechanism by which UVC irradiated bacterial cells are rendered more sensitive to thermal processing. In this study, the combinatorial use of UVC followed by FP was used to improve the efficiency of *L. innocua* inactivation by FP.

L. innocua has been used as a surrogate microorganism in previous pilot-plant scale studies for FP decontamination of frankfurters (Kozempel and others 2000; Sommers and others 2002, 2008; Murphy and others 2005a, 2006). FP conditions (1.5 s steam, 121 °C) were used based on previous research and conditions of use in actual current commercial practice (personal communication, Seth Pulsfus, Alkar-RapidPak Inc.). FP treatments of 0.75, 1.5, and 3 s at 121 °C resulted in log reductions of 1.99 (± 0.11), 1.97 (± 0.13), or 2.51 (± 0.06), respectively (Table 1). The reduction at 3 s FP was significantly greater than those at 0.75 or 1.5 s as determined by ANOVA ($n = 3$, $\alpha = 0.05$), although it should be noted that FP at 3 s would be too slow for commercial processing and packaging of frankfurters. UVC treatment of frankfurters at doses of 1, 2, or 4 J/cm^2 achieved inactivations of 1.47 (± 0.17), 1.48 (± 0.18), or 1.53 (± 0.13), respectively (Table 1). There was no statistical difference in the log reductions at the different UVC doses as determined by ANOVA ($n = 3$, $\alpha = 0.05$). UVC followed by FP (1 J/cm^2 : 0.75 s to 4 J/cm^2 : 3 s) resulted in a 3.19 to 3.89 log reduction of *L. innocua*. Although the log reductions qualitatively increased with increasing UV and FP exposures, statistically they were similar, although significantly greater than UVC or FP when used singly (Table 1). These results indicate an additive effect of using UVC prior to the FP process as noted by Sommers and Geveke (2006), who used UVC followed by the Vacuum–steam–vacuum process to improve the inactivation of *Listeria*.

Table 1—Inactivation (log CFU/g) of *L. innocua* on frankfurters by UVC and FP (shaded cells indicate combined UVC-FP processing).

	0 J/cm²	1 J/cm²	2 J/cm²	4 J/cm²
0 s	0.00 (± 0.00)	1.47 (± 0.17)[a]	1.48 (± 0.18)[a]	1.53 (± 0.13)[a]
0.75 s	1.99 (± 0.11)[b]	3.19 (± 0.08)[d]	3.39 (± 0.08)[d]	3.22 (± 0.17)[d]
1.5 s	1.97 (± 0.13)[b]	3.51 (± 0.05)[d]	3.47 (± 0.17)[d]	3.38 (± 0.19)[d]
3 s	2.51 (± 0.06)[c]	3.64 (± 0.20)[d]	3.56 (± 0.12)[d]	3.89 (± 0.18)[d]

Each experiment was conducted independently 3 times. Values listed by the same letter are statistically similar as determined by ANOVA ($n = 3$, $\alpha = 0.05$). All treatment conditions indicated significant increases in microbial inactivation over the levels in the untreated controls (no FP, no UVC).

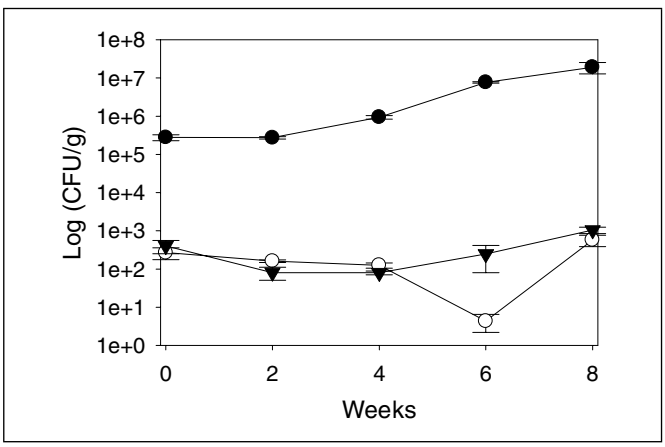

Figure 1—Growth of *L. innocua* on frankfurters (8 °C) treated with UVC light and flash pasteurization. Untreated controls are shown as closed circles, 1 J/cm² UVC : 1.5 s FP (triangles) and 2 J/cm² UVC : 3 s FP (open circles). Standard error of the mean shown as error bars ($n = 3$).

Table 2—Effect of UVC and FP on frankfurter quality.

	Untreated control	1 J/cm² UV 1.5 s FP	2 J/cm² UV 3 s FP
a-value	13.59 (± 0.91)[a]	13.79 (± 0.78)[a]	11.52 (± 1.10)[b]
b-value	27.84 (± 1.51)[a]	27.35 (± 1.09)[a]	27.05 (± 1.90)[a]
L-value	57.60 (± 0.53)[a]	50.11 (± 0.66)[b]	50.72 (± 0.46)[b]
Texture	1836 (± 120)[a]	1860 (± 334)[a]	1714 (± 87.0)[a]

Each experiment was conducted independently 3 times ($n = 3$). Different letters in rows indicate statistical difference as determined by ANOVA ($n = 3$, $\alpha = 0.05$).

To examine the effect of UVC and FP on the proliferation of *L. innocua* during long-term refrigerated storage, an 8-wk storage study (8 °C) was performed using untreated frankfurters that contained SDA/PL, those treated with 1 J/cm^2 : 1.5 s and 2 J/cm^2 : 3 s UVC and FP. On the untreated frankfurters, growth of *L. innocua* was inhibited for approximately 2 wk at 10 °C, but the population rose to $>10^7$ CFU/g by the end of the storage period (Figure 1). This data agrees with that obtained in a previous study that examined the ability of *L. monocytogenes* to proliferate in beef bologna that contained PL and SDA mixtures at an abuse temperature of 10 °C in which growth of the pathogen was inhibited for only 2 to 4 wk (Sommers and others 2003). In contrast, UVC followed by FP resulted in >3.2 log reduction of *L. innocua*, but the growth of the survivors did not occur for 8 wk storage at 8 °C (Figure 1). There was no advantage to using 1 J/cm^2 : 1.5 s against 2 J/cm^2 : 3 s (Figure 1), which is in agreement with the log reduction data shown in Table 1.

Previous research has indicated that UVC (up to 4 J/cm^2) and FP (3 s) have no effect on frankfurter color and texture (Sommers and Geveke 2006; Sommers and others 2008a). To evaluate the effect of FP on frankfurter quality, color and texture analysis were conducted (Table 2). Only the reduction in *L*-value was statistically significant for the UVC and FP combination as determined by ANOVA ($n = 3$, $\alpha = 0.05$), but this was visually unnoticeable. Maximum shear-force (g) was not affected by FP and UVC combination as determined by ANOVA ($n = 3$, $\alpha = 0.05$). There was no effect of FP on either frankfurter color or shear force as determined by ANOVA ($n = 3$, $\alpha = 0.05$).

Conclusions

Low numbers or elimination of *Listeria* in frankfurters, which can be achieved through the use of intervention technologies in combination with antimicrobials, equates to a low risk of listeriosis (Chen and others 2003). The results of this study indicate that the combination of UVC and FP, when used with antimicrobial compounds, would be a commercially realistic and effective low cost method for controlling *Listeria* on frankfurters.

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